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## REPRODUCTIVE BIOLOGY AND THE PROCESS OF DOMESTICATION OF THE COLUMNAR CACTUS *STENOCEREUS STELLATUS* IN CENTRAL MEXICO<sup>1</sup>

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Pollination biology, breeding system, and floral phenology of the columnar cactus *Stenocereus stellatus* were studied in wild, wild managed in situ and cultivated populations of central Mexico, in order to examine whether these aspects have been modified under domestication and whether they determine reproductive barriers between wild and manipulated individuals. Individuals of both wild and manipulated populations are self-incompatible, indicating that artificial selection has not modified the breeding system. Their pollination biology is also similar. Anthesis is mainly nocturnal, with a peak of nectar production between 0200 and 0400 when the stigma presents maximum turgidity. Nocturnal visitors are the effective pollinators. Nearly 75% of flowers exposed for nocturnal pollination set fruit, while none of the flowers exposed for diurnal pollination produced fruits. The bats *Leptonycteris curasoae*, *L. nivalis*, and *Choeronycteris mexicana* (Glossophaginae) are the most likely pollinators, and their time of foraging is synchronized with the time of nectar production and stigma receptivity in *S. stellatus*. Bats potentially move pollen over a considerable distance, so there is apparently no spatial isolation to prevent pollen exchange between wild and cultivated populations. Phenological studies showed that there are also no apparent temporal barriers. However, manual cross pollination failed between some domesticated and wild phenotypes, suggesting that gene flow between wild and cultivated populations might be limited by pollen incompatibility.

**Key words:** bat pollination; Cactaceae; columnar cacti; domestication; Mixteca; reproductive biology; *Stenocereus stellatus*; Tehuacán Valley.

*Stenocereus stellatus* (Pfeiffer) Riccobono is a columnar cactus endemic to central Mexico. Its range is centered on the Tehuacán Valley and La Mixteca Baja, which are located in the southeast of Puebla, the northwest of Oaxaca, and the northeast of Guerrero (Fig. 1). This species occurs in the wild as part of the tropical deciduous and thorn-scrub forests, but it is also cultivated and managed in situ because of its edible fruits. According to Casas et al. (1997), cultivation of *S. stellatus* is widely practiced in home gardens, whereas management in situ of wild populations is generally associated with clearance of natural vegetation for cultivation of maize. Both cultivation and management in situ are important activities practiced by the Mixtec, Nahuatl, and Popoloca indigenous people of the region for the direct consumption and commercialization of fruits.

Most commonly, people cultivate *S. stellatus* by vegetative propagation of stem segments of previously cultivated individuals but sometimes also of wild individuals,

which are selected according to their morphological characteristics. However, people also tolerate seedlings of this species in home gardens and leave the decision of sparing or removing the individual plants after these reach the reproductive stage, when quality of their fruit is evident. Management in situ of wild populations is a form of silvicultural management of *S. stellatus*. In this case, people selectively spare desirable individuals and remove others during clearance of vegetation (Casas et al., 1997; Casas, Valiente-Banuet, and Caballero, in press).

Although vegetative propagation via stem segments is common in wild populations, these populations present considerable amounts of morphological and genetic variation, suggesting that sexual reproduction plays also an important role in the propagation of *S. stellatus* (Casas, 1997; Casas et al., 1997). Wild managed in situ populations are less variable than the wild ones because people spare only part of the individuals that constituted the original wild populations (Casas, 1997). But variation is relatively high within cultivated populations because people continually replace individual plants by introducing stem segments of both wild and cultivated individuals and including materials from other villages or regions, but also because people continually allow the recruitment of new plants derived from seeds dispersed via bat, bird, or human feces within home gardens (Casas, 1997).

Casas (1997) found that wild, wild managed in situ, and cultivated populations significantly differ in morphology, especially in fruit characters such as fruit size,

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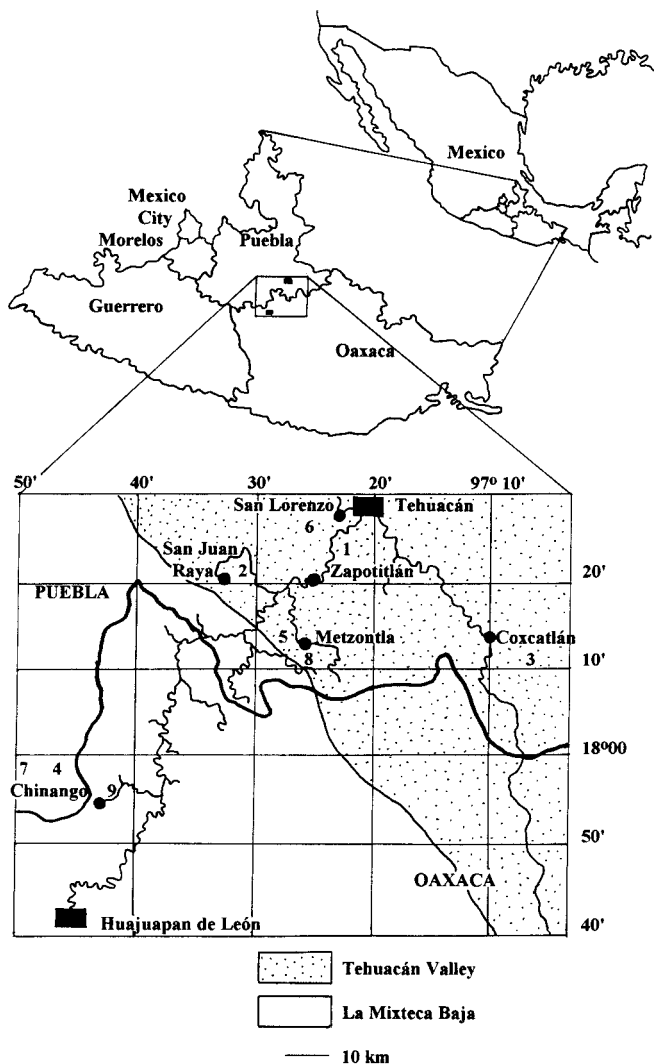


Fig. 1. Range of *Stenocereus stellatus* and populations studied. Wild populations: 1 = Zapotitlán-W; 2 = San Juan Raya-W; 3 = Coxcatlán-W; 4 = Chinango-W. Populations managed in situ: 5 = Metzontla-M; 6 = San Lorenzo-M; 7 = Chinango-M. Cultivated populations: 8 = Metzontla-C; 9 = Chinango-C.

density of areoles in fruit peel, and seed number and size, among others. Because fruit characters are the main targets of human selection and because they significantly differ according to the method of management of the populations, Casas (1997) suggested that artificial selection is causing significant effects in the phenotypic structure of populations and that, therefore, domestication, and not merely management in situ and cultivation, has taken place in this species. Artificial selection could have been operating on *S. stellatus* over long periods of time, as suggested by archaeological information on the antiquity of its use. Thus, in the Tehuacán Valley, MacNeish (1967) and Smith (1967) identified remains of *S. stellatus* in caves inhabited by humans from strata 5500 yr BP.

Ethnobotanical and morphological studies (Casas, 1997; Casas et al., 1997) found that, at present, artificial selection is directed to favor individuals producing large fruits, sweet pulp with colors other than red, and thin peel

with few areoles. Artificial selection is carried out mainly by keeping and enhancing through vegetative propagation the desirable phenotypes and by eliminating the undesirable ones. This process seems to be the basic mechanism that maintains and develops the morphological divergence between wild and cultivated populations (Casas, 1997). As a result of such divergence, some phenotypes characterized by large fruits (up to 80 cm<sup>3</sup>), pink, purple, yellow, orange, or white pulp and fruit peel with few areoles occur commonly in home gardens, but they are rare or absent in wild populations (Casas, 1997; Casas et al., 1997). This suggests either that these variants are reproductively isolated from wild populations and/or that natural selection against these phenotypes has occurred in the wild. The general purpose of this study was, thus, to examine factors influencing the maintenance of morphological divergence between wild and cultivated populations of *S. stellatus*.

Pollination biology and the breeding system of this species were analyzed in order to assess whether these aspects have been modified by domestication and whether they could reduce or prevent gene flow between wild and cultivated populations.

Although Gibson and Horak (1978) considered that the flowers of *S. stellatus* are pollinated by hummingbirds, preliminary observations by Casas (1997) indicated that anthesis occurs at night, when the flowers produce an unpleasant scent, suggesting that flowers of this species are chiropterophilic as defined by Faegri and van der Pijl (1979) and Rowley (1980). Accordingly, aspects of the floral biology of *S. stellatus* were investigated in order to clarify the most likely pollinators and mechanisms of pollination.

Breeding system studies have been carried out for nearly 20 of 70 species of columnar cacti of the tribe Pachycereeae. Among the most relevant studies are those by McGregor et al. (1959, 1962), Alcorn et al. (1959) and Alcorn, McGregor, and Olin (1961, 1962), Fleming et al. (1994), and Fleming, Tuttle, and Horner (1996) with the saguaro *Carnegiea gigantea* (Engelman) Britton & Rose, the organpipe *Stenocereus thurberi* (Engelman) Britton & Rose, and *Pachycereus pringlei* (S. Watson) Britton & Rose. Also important are studies by Soriano, Sosa, and Rosell (1991), Sosa and Soriano (1992), and Nassar, Ramírez, and Linares (1997) with *Stenocereus griseus* (Haworth) Buxbaum, *Subpilocereus repandus* Schum., *S. horrispinus*, *Pilosocereus tillianus* Schum., *P. moritzianus*, and *P. lanuginosus* in Venezuela, as well as those carried out in the Tehuacán Valley by Valiente-Banuet et al. (1996, 1997a, b) with *Pilosocereus chrysacanthus* (Weber) Britton & Rose, *Neobuxbaumia macrocephala* (Weber) Daws, *N. mezcalaensis* (Bravo-Hollis) Backeb., *N. tetetzo* (Weber) Backeb., and *Pachycereus weberi* (Coulter) Backeb.

These studies have shown that self-pollination is ineffective in all cases, with some exceptions found in extratropical species in the Sonoran Desert. Therefore, it was possible to expect that wild populations of *S. stellatus* would be outcrossed and probably incapable of effective self-pollination. However, this pattern of reproduction might have been modified in cultivated populations, as in other species, such as apples, pears, and other perennial, self-incompatible and vegetatively propagated

TABLE 1. General information on populations of *Stenocereus stellatus* sampled.

| Population      | Ecological status | Elevation (m) | Annual mean temp. (°C) | Annual mean rain fall (mm) | Origin of soils | Habitat                   | No. of individuals included in breeding system studies | No. of individuals included in phenological studies | Sampled area in phenological studies (m <sup>2</sup> ) |
|-----------------|-------------------|---------------|------------------------|----------------------------|-----------------|---------------------------|--------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------|
| <b>Tehuacán</b> |                   |               |                        |                            |                 |                           |                                                        |                                                     |                                                        |
| Zapotitlán-W    | wild              | 1550          | 21.2 <sup>a</sup>      | 450.0 <sup>a</sup>         | sandstones      | thorn-scrub forest        | 10                                                     | 32                                                  | 1500                                                   |
| S. J. Raya-W    | wild              | 1800          | 20.9 <sup>a</sup>      | 649.7 <sup>a</sup>         | limestones      | thorn scrub-forest        | 3                                                      | 7                                                   | 8500                                                   |
| Coxcatlán-W     | wild              | 1000          | 23.8 <sup>a</sup>      | 440.6 <sup>a</sup>         | volcanic        | tropical deciduous forest | 9                                                      | 14                                                  | 6000                                                   |
| Metzontla-M     | managed in situ   | 2000          | 17.2 <sup>b</sup>      | 527.9 <sup>b</sup>         | limestones      | agricultural area         | 8                                                      | 12                                                  | 1000                                                   |
| S. Lorenzo-M    | managed in situ   | 1700          | 19.1 <sup>a</sup>      | 590.0 <sup>a</sup>         | limestones      | fallow fields             | 5                                                      | 23                                                  | 10000                                                  |
| Metzontla C     | cultivated        | 1900          | 17.2 <sup>a</sup>      | 527.9 <sup>a</sup>         | alluvial        | home gardens              | 10                                                     | 26                                                  | 2570                                                   |
| <b>Mixteca</b>  |                   |               |                        |                            |                 |                           |                                                        |                                                     |                                                        |
| Chinango-W      | wild              | 1700          | 20.6 <sup>a</sup>      | 720.5 <sup>a</sup>         | volcanic        | tropical deciduous forest | 6                                                      | 14                                                  | 6000                                                   |
| Chinango-M      | managed in situ   | 1700          | 20.6 <sup>a</sup>      | 720.5 <sup>a</sup>         | volcanic        | agricultural area         | 5                                                      | 11                                                  | 7500                                                   |
| Chinango-C      | cultivated        | 1600          | 20.6 <sup>a</sup>      | 720.5 <sup>a</sup>         | alluvial        | home gardens              | 15                                                     | 56                                                  | 17000                                                  |

<sup>a</sup> Based on García, 1988.<sup>b</sup> Based on Valiente, 1991.

plants, in which mutants to self-compatibility sometimes have been favored by human selection (see Proctor, Yeo, and Lack, 1996). One of the advantages of inbreeding plants of such species is that they can produce fruit and seed reliably in the absence of any pollinator, or in conditions that do not favor the pollinator. Inbreeding also eliminates dependence on sources of compatible pollen, which could be especially important in plants that, like *S. stellatus*, are vegetatively propagated. This study was thus directed to determine whether sexual reproduction in this plant species involves inbreeding, outbreeding, or both, and whether the reproduction system has been modified in cultivated populations by processes of domestication.

Temporal patterns of production of flowers and fruits of wild plants are commonly modified by domestication, in order to adjust them to human convenience. This might have occurred in *S. stellatus* and might be a possible factor causing reproductive barriers between wild and cultivated populations. Accordingly, a comparative analysis of floral phenology was carried out in wild, managed in situ, and cultivated populations in the Tehuacán Valley and La Mixteca Baja in order to examine whether natural or artificial selection had affected the time of production of flowers and whether this had caused significant barriers for pollen exchange between wild and cultivated populations and between populations of different geographic areas.

Pollen incompatibility in crosses between wild and domesticated types of plant species is another possible cause of reproductive isolation influencing the process of domestication. In this study, experiments were carried out in order to test the possible occurrence of pollen incompatibility between some wild and cultivated phenotypes of this species.

## MATERIALS AND METHODS

**Study area**—The research was carried out in the Tehuacán Valley, where *Stenocereus stellatus* occurs at altitudes from 1220 to 2000 m and between 300 and 650 mm of precipitation per year, and in La Mixteca Baja, where *S. stellatus* is distributed in areas at altitudes from

600 to 1800 m with precipitation between 600 and 800 mm per year. In both areas the annual mean temperature ranges from 17° to 26°C.

Nine populations were included in this study (Fig. 1). Four of these populations are wild (Zapotitlán-W, San Juan Raya-W, and Coxcatlán-W in the Tehuacán Valley and Chinango-W in La Mixteca Baja); three are managed in situ (Metzontla-M and San Lorenzo-M in the Tehuacán Valley and Chinango-M in La Mixteca Baja); and two are cultivated (Metzontla-C in the Tehuacán Valley and Chinango-C in La Mixteca Baja). General information on environmental conditions of these populations is summarized in Table 1.

**Floral biology**—Time of anthesis, discharge of pollen from anthers, turgidity of stigma, and closing of flowers were observed at 30-min intervals from 1900 to 1000 on flowers from wild (Zapotitlán-W and Coxcatlán-W) and cultivated (Metzontla-C) populations in the Tehuacán Valley, as well as from wild (Chinango-W) and cultivated (Chinango-C) populations in La Mixteca Baja. Five flowers were observed each night, and one night was spent on each population.

Amounts of nectar produced during anthesis were measured in a wild population (Zapotitlán-W) and a cultivated population (Chinango-C). In each population, flowers were covered with mosquito net exclusion bags before anthesis in order to avoid consumption of nectar by visitors. Three flowers were collected every hour from 2300, when flowers had just opened, to 0700, when flowers started to close. Amounts of nectar were measured by using calibrated capillary tubes. Sugar concentration (percentage sucrose) was measured using ten randomly chosen flowers that were bagged before anthesis. Measures were carried out at 0300 in samples of the nectar accumulated with a hand-held refractometer (American Optical No. 9103).

**Breeding systems**—Plants in Zapotitlán-W, Coxcatlán-W, and Chinango-C populations were studied during July 1995. Flower buds were labeled and covered with exclusion bags the afternoon before anthesis. Based on methods developed by Valiente-Banuet et al. (1996) for studying breeding systems of *Neobuxbaumia tetetzo* in Tehuacán, six experimental treatments were applied to at least ten flowers per treatment. The treatments were as follows: (1) nonmanipulated self-pollination, in which bagged flowers were followed until they either aborted or produced mature fruits; (2) manual self-pollination, in which bagged flowers were hand-pollinated with their own pollen using a paint brush at 0300; (3) cross-pollination, in which flowers were hand-pollinated with pollen collected from other individuals of the same population located at least 300 m away from the pollinated plants (flowers were pollinated at 0300 using a paint brush to transfer the pollen); (4) nocturnal polli-



nation, in which flowers were exposed to nocturnal visitors by removing the bags between 2200 (after sunset but before anthesis) and 0630 (just before sunrise); (5) diurnal pollination, in which flowers were exposed to diurnal visitors from 0700 (sunrise) to 1000 (after closure of flowers); and (6) natural pollination, in which a sample of unbagged flowers was marked and followed until they either aborted or produced mature fruits. The total number of aborted flowers, mature fruits, and seeds per fruit in each treatment were counted.

In addition, individuals in the other six populations (San Juan Raya-W, Chinango-W, Metzontla-M, San Lorenzo-M, Chinango-M, and Metzontla-C populations) were tested for nonmanipulated self-pollination, and flowers from the same individuals were also manually self-pollinated. In each of the population, all pollinations were carried out during one night in the peak of the flowering season. Total number of individuals per population included in this study are presented in Table 1. Number of individuals and flowers per treatment are indicated in Table 2.

**Flower visitors**—Mist nets were used to capture flower visitors. A total of three nets per population (3 m high and 12 m long, separated by ~100 m) were set up on a random transect. They were placed alongside or underneath flowering *S. stellatus* in the wild populations Zapotitlán-W (five nights) and Coxcatlán-W (four nights) and in the cultivated population Chinango-C (four nights) between May and September 1995. Nets were set up from 2000 (before anthesis), left until 1000 (just after flower closure), and visited continually. A total of 39 nets were set up and 546 netting-hours were accomplished. The species captured were identified and the hour of capture recorded.

Insects were captured by approaching jars to flowers or by forceps when the insects were inside the flowers and then killed in a vial containing a piece of blotting paper with ethyl acetate. Insects were pinned onto entomological pins and identified. Attempts to capture insects were conducted every 2 h from 2300 to 1000 the same nights that netting was carried out.

Pollen samples were collected from bodies of the animals captured by using small squares of fuchsin jelly (Beattie, 1971). These fuchsin squares were mounted on a microscope slide and melted by using a candle. The slide was scanned for pollen grains of *Stenocereus stellatus*.

**Dynamics of flowering in populations**—The numbers of individuals flowering every 2-4 wk between April and November 1995 in samples of all nine populations were counted. Wild and managed in situ populations were sampled by transects including the longest dimension of the population per 5 m wide. Approximately 10% of the home gardens in a village were sampled at random, but only different clones identified by local people were considered in the study. Individuals sampled were marked by metallic labels and numbers drawn with waterproof ink. The sampled area and the number of individuals studied per population are presented in Table 1. A long stick with a mirror at the top was used to enable counts to be made from the crowns of tall branches.

The percentage of individuals per population producing flowers was recorded. The data recorded were processed as follows: (1) date of appearance of first flowers (Dafni, 1992); (2) date when 25% of individuals showed open flowers; (3) date when 25-50% of individuals showed open flowers; (4) date when 50% or more of individuals showed open flowers; (5) date when 25-50% of individuals showed open flowers, and the rest were already withered; (6) date when <25% of individuals were flowering; (7) date when <10% of the individuals were flowering; and (8) date when flowering finished (Dafni, 1992). These data were also used to define the peak of flowering (date of maximum number of flowers per individual and of flowering plants per population) and flowering duration (flowering duration of the sample in days) (Dafni, 1992). Number of flowering days and number of flowers at flowering peak per individual were compared by ANOVA among wild, wild managed in situ, and cultivated populations within the Tehuacán Valley and within La Mixteca.

Experiments were carried out for testing the occurrence of pollen

TABLE 2. Numbers of fruits and seeds produced following different types of pollination in nine populations of *Stenocereus stellatus*. Different bold face letters indicate ranges of numbers of seeds that differ significantly within populations according to the Tukey 95% highest significant difference test.

| Population   | Self-pollination |     |     |    |    |     |        |    |          |          |    |     |    |    |          |    |            |          | Manual cross-pollination |          |            |          |    |          | Diurnal pollination |    |     | Nocturnal pollination |    |     | Natural pollination |  |  |
|--------------|------------------|-----|-----|----|----|-----|--------|----|----------|----------|----|-----|----|----|----------|----|------------|----------|--------------------------|----------|------------|----------|----|----------|---------------------|----|-----|-----------------------|----|-----|---------------------|--|--|
|              | Nonmanipulated   |     |     |    |    |     | Manual |    |          |          |    |     |    |    |          |    |            |          |                          |          |            |          |    |          |                     |    |     |                       |    |     |                     |  |  |
|              | NI               | NF  | %FS | NI | NF | %FS | NI     | NF | %FS      | NI       | NF | %FS | NI | NF | %FS      | NI | NF         | %FS      | NI                       | NF       | %FS        | NI       | NF | %FS      | NI                  | NF | %FS | NI                    | NF | %FS |                     |  |  |
| Zapotitlán-W | 10               | 37  | 0   | 10 | 10 | 0   | 9      | 10 | 60       | 290 ± 48 | 9  | 10  | 0  | 9  | 10       | 0  | 9          | 10       | 80                       | 951 ± 24 | 10         | 20       | 75 | 934 ± 35 |                     |    |     |                       |    |     |                     |  |  |
| Coxcatlán-W  | 9                | 20  | 0   | 9  | 10 | 0   | 9      | 10 | 50       | 342 ± 27 | 9  | 10  | 0  | 9  | 10       | 0  | 9          | 10       | 70                       | 703 ± 33 | 9          | 20       | 65 | 750 ± 15 | <b>B</b>            |    |     |                       |    |     |                     |  |  |
| Chinango-C   | 15               | 35  | 0   | 15 | 20 | 0   | 9      | 10 | 60       | 689 ± 52 | 9  | 15  | 0  | 9  | 20       | 75 | 1540 ± 125 | 10       | 30                       | 73       | 1472 ± 227 | <b>B</b> |    |          |                     |    |     |                       |    |     |                     |  |  |
| S. J. Raya-W | 3                | 13  | 0   | 3  | 10 | 0   | —      | —  | —        | <b>A</b> | —  | —   | —  | —  | —        | —  | —          | —        | —                        | —        | —          | —        | —  |          |                     |    |     |                       |    |     |                     |  |  |
| Chinango-W   | 6                | 22  | 0   | 6  | 7  | 0   | —      | —  | —        | —        | —  | —   | —  | —  | —        | —  | —          | —        | —                        | —        | —          | —        | —  |          |                     |    |     |                       |    |     |                     |  |  |
| Metzontla-M  | 8                | 15  | 0   | 8  | 6  | 0   | —      | —  | —        | —        | —  | —   | —  | —  | —        | —  | —          | —        | —                        | —        | —          | —        | —  |          |                     |    |     |                       |    |     |                     |  |  |
| S. Lorenzo-M | 5                | 20  | 0   | 5  | 15 | 0   | —      | —  | —        | —        | —  | —   | —  | —  | —        | —  | —          | —        | —                        | —        | —          | —        | —  |          |                     |    |     |                       |    |     |                     |  |  |
| Chinango-M   | 5                | 15  | 0   | 5  | 10 | 0   | —      | —  | —        | —        | —  | —   | —  | —  | —        | —  | —          | —        | —                        | —        | —          | —        | —  |          |                     |    |     |                       |    |     |                     |  |  |
| Metzontla-C  | 10               | 30  | 0   | 10 | 10 | 0   | —      | —  | —        | —        | —  | —   | —  | —  | —        | —  | —          | —        | —                        | —        | —          | —        | —  |          |                     |    |     |                       |    |     |                     |  |  |
| Total        | 71               | 207 | 0   | 71 | 98 | 0   | 27     | 30 | 17 (57%) | 27       | 35 | 0   | 27 | 40 | 30 (75%) | 29 | 70         | 50 (71%) |                          |          |            |          |    |          |                     |    |     |                       |    |     |                     |  |  |

Note: NI = number of individuals; NF = number of flowers in treatments; % FS = % of fruit set; NS = mean number of seeds ± SE per successful fruit in treatments.

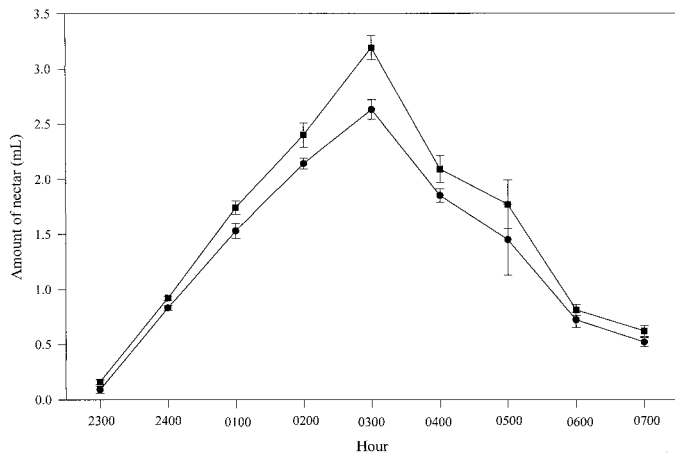


Fig. 2. Production of nectar during anthesis in individuals of *Stenocereus stellatus* from the wild population Zapotitlán-W (●) and the cultivated population Chinango-C (■) ( $N = 3$  flowers per hour registered per population).

incompatibility between cultivated phenotypes and wild individuals. Pieces of branch tops with flower buds, to open the night the experiment were conducted, were cut from individuals of the “blanco” (white pulp), “amarillo” (yellow pulp), “morado” (purple pulp), and “colorado” (red pulp) cultivated variants from Chinango, and these were transported to wild population. Flower buds managed in this way continue their normal development until anthesis. Pollen from flowers of the cultivated variants selected was manually deposited in stigmata of flower sets of wild individuals. A treatment including pollen from flowers of a wild individual was also made. A total of ten bagged flowers per treatment (50 flowers from 19 wild individuals) were included. Manual pollination was conducted at 0300 during the peak of flowering by using paint brushes. The total number of aborted and successful fruits was recorded and compared between treatments.

## RESULTS

**Floral biology**—Anthesis began ~2000 when the outermost whorl of tepals began to separate. One hour later the middle whorl started also to open and at ~2200 the innermost whorl did the same. Around 2300 the flowers were completely open, but the stamens were inclined towards the center of the flower and the stigma lobes were closed together, flaccid, and enclosed by the stamens. One hour later (~2400) the stamens became vertical and pollen started being released. By this time, the stigma lobes had already started to open and they became turgid slowly, but were not completely open until 2 or 3 h later (~0200 and 0300). Flowers of *S. stellatus* opened through the night and started to close ~0700 when stamens and stigma lobes inclined again towards the center of the flower and the whorls of tepals started to contract. Around 0800 the innermost whorl was closed, and 1 h later the whole flower was completely closed.

Figure 2 shows the total amount of nectar produced by flowers at different hours throughout anthesis. Rates of nectar production were similar in the two populations studied. The peak of production coincided with the time when the stigma lobes were completely open and turgid. Concentration of nectar averaged  $18.3 \pm 0.75\%$  (mean  $\pm$  SD,  $N = 5$ ).

These observations indicate that anthesis is predomi-

nantly nocturnal; the nocturnal visitors have the opportunity to visit flowers for 7–8 h, whereas the diurnal visitors have only 1 or 2 h. Both nocturnal and diurnal visitors have the opportunity to obtain pollen and nectar, although these resources are particularly abundant during the night. This suggests that pollination most probably occurs from 0200 to 0500, when maximum turgidity of the stigma lobes coincides with the peak of production of nectar.

**Breeding systems**—Results from experiments on breeding systems (Table 2) indicate that both nonmanipulated and manual self-pollination did not produce any fruit set. On the contrary, in all the populations studied, manual cross-pollination produced fruits and seeds although fewer than in treatments of natural pollination. The results also indicate that successful pollination was achieved by nocturnal visitors, while no fruit set resulted from diurnal visits. The production of fruits and seeds obtained from nocturnal pollination was similar to that obtained from natural pollination.

**Flower visitors**—Table 3 lists the species of bats, birds, and hawk moths captured in mist nets, as well as insects captured during their visits to flowers. Hawk moths, bats, and beetles were observed actively interacting with flowers of *S. stellatus* at night. In all cases, beetles were observed destroying both stamens and pistils. Although beetles are known to be destructive pollinators in other plant species (see Proctor, Yeo, and Lack, 1996), they appeared not to be indispensable pollinators of *S. stellatus*, since they were absent in the flowers that produced fruits in the tests of nocturnal pollination. Hawk moths were observed flying close to the flowers with their proboscises extended, apparently taking nectar from the flowers of *S. stellatus*, but without making significant contact with the stigma or anthers, although pollen grains of *S. stellatus* were identified on the bodies of some of the hawk moths captured (Table 3). After taking nectar from a flower, hawk moths generally visited other flowers close to it, from other branches of the same individual or from neighboring individuals. Captures of hawk moths occurred from 2100 to 0100.

Bats were observed entering the flowers so that their faces and sometimes even their shoulders became covered with pollen. They invariably touched the stigma surface when seeking nectar. Pollen of *S. stellatus* was identified on the bodies of all the bats captured, and Glossophaginae species were by far the most frequent animals captured presenting pollen of *S. stellatus* (Table 3). Bats were observed taking nectar from one or two flowers of the same branch and then flying away. Captures of bats occurred from 2000 to midnight and then from 0200 to 0500, suggesting two periods of activity through the night.

Diurnal visitors contacted both anthers and stigma, although by the time they visited the flowers the stigmas were flaccid and apparently not receptive.

**Dynamics of flowering in populations**—Table 4 shows that flowering occurred over a period of at least 91 d, with a few flowers blooming per individual every night. Accordingly, the date of flowering commencement and

TABLE 3. Visitors to flowers of *Stenocereus stellatus*.

| Group   | Species                                                    | Habit             | No. bodies sampled | No. bodies with pollen of <i>S. stellatus</i> |
|---------|------------------------------------------------------------|-------------------|--------------------|-----------------------------------------------|
| Birds   | <i>Amazilia violiceps</i> Gould (Trochilidae)              | Diurnal           | 1                  | 1                                             |
|         | <i>Cynanthus latirostris</i> Gould (Trochilidae)           | Diurnal           | —                  | —                                             |
|         | <i>Cynanthus sordidus</i> Gould (Trochilidae)              | Diurnal           | 1                  | 1                                             |
|         | <i>Melanerpes hypopolius</i> Wagler (Picidae)              | Diurnal           | 1                  | 1                                             |
| Bats    | <i>Leptonycteris curasoae yerbbae</i>                      | Nocturnal         | 33                 | 33                                            |
|         | Martínez y Villa (Glossophaginae)                          |                   |                    |                                               |
|         | <i>Leptonycteris nivalis</i> Saussure (Glossophaginae)     | Nocturnal         | 11                 | 11                                            |
|         | <i>Choeronycteris mexicana</i> Tshudi (Glossophaginae)     | Nocturnal         | 37                 | 37                                            |
|         | <i>Artibeus jamaicensis</i> J. A. Allen (Sternodermatinae) | Nocturnal         | 4                  | 4                                             |
|         |                                                            |                   |                    |                                               |
| Insects | <i>Apis mellifera</i> L. Hymenoptera (Apidae)              | Diurnal           | —                  | —                                             |
|         | <i>Melipona</i> sp. Hymenoptera (Apidae)                   | Diurnal           | —                  | —                                             |
|         | <i>Trigona</i> sp. Hymenoptera (Apidae)                    | Diurnal           | —                  | —                                             |
|         | <i>Musca domestica</i> L. Diptera                          | Diurnal           | —                  | —                                             |
|         | <i>Bombus</i> spp. Hymenoptera (Apidae)                    | Diurnal           | —                  | —                                             |
|         | <i>Eumorpha</i> sp. (Lepidoptera)                          | Nocturnal         | 9                  | 5                                             |
|         | Hawk moth sp. 2                                            | Nocturnal         | 1                  | 1                                             |
|         | <i>Carpophylus</i> sp. 1 (Coleoptera)                      | Diurnal/nocturnal | 3                  | 3                                             |
|         | <i>Carpophylus</i> sp. 2 (Coleoptera)                      | Diurnal/nocturnal | 5                  | 5                                             |
|         |                                                            |                   |                    |                                               |

termination may differ by more than one month between populations. However, the peak of the flowering period in all populations occurred between the first and third week of July. At flowering peak, individuals of cultivated populations presented significantly more flowers in anthesis than individuals of the rest of the populations of each region. Flowering duration in individuals of cultivated populations was also significantly longer than in individuals of any other population (Table 4). However, the flowering periods in different populations overlapped by at least 75 d. This indicates that temporal barriers for pollination between populations are unlikely.

**Crosses between cultivated and wild phenotypes**—All fruits from crosses tested with pollen from the four cultivated phenotypes aborted, while 60% of crosses with pollen from wild individuals produced successful fruits.

## DISCUSSION

Our studies of floral biology confirmed that anthesis in *Stenocereus stellatus* is mainly nocturnal with the maximum offer of nectar and stigma receptivity occurring at night. Experiments showed that *S. stellatus* cannot produce seeds in the absence of pollinators and that nocturnal visitors were the only effective pollinators. Of the nocturnal visitors, bats were the most frequent visitors of flowers of *S. stellatus*. Pollen of this plant species was found on all the bodies of bats examined, and bats were active during the time when stigmata are receptive. This indicates that bats are the most probable pollinators of *S. stellatus*.

Hawk moths could have some role in pollination of this plant species because they visit flowers of *S. stellatus* and are active during the time when stigmata are receptive. However, the period of activity of hawk moths (2100–0100) seems to include only a small fraction of the period of receptivity of stigmata, and their bodies seldom touch the flowers. In addition, hawk moths seem

to move over short distances, which may increase the probability of pollen being moved from flower to flower on the same individual, hence pollination probably being ineffective. Consequently, it would be expected that hawk moths are significantly less effective pollinators of *S. stellatus* than bats. Further experiments excluding alternately bats and hawk moths from flowers could prove this hypothesis.

Diurnal visitors seem unlikely to be pollinators of *S. stellatus*, unless the stigma remains receptive until the morning and flowers have not been visited by bats during the previous night. However, neither the present study nor those undertaken by Valiente-Banuet et al. (1997a, b) in the region with other species of columnar cacti showing nocturnal anthesis have recorded any fruit production by diurnal visitors.

Three species of specialized nectarivorous bats, *Leptonycteris curasoae*, *L. nivalis*, and *Choeronycteris mexicana* (Fleming, 1993; Fleming, Nuñez, and Da Silveira, 1993), participate in the pollination of *Stenocereus stellatus*. Valiente-Banuet et al. (1996) observed that on different nights from April to June in the Tehuacán Valley, *L. curasoae* and *C. mexicana* presented two peaks of nocturnal activity, one occurring between 1900 and 2300 and the other between 0100 and 0500. In general, information from the present study, obtained from July to September, agrees with this observation. During the first peak of bat activity pollen is shed, whereas during the second peak the maximum production of nectar and stigma turgidity occur. This suggests that during the first peak, bats would be taking part of the nectar from the flowers and they become covered with pollen. During the second peak of activity, new visits to flowers would be carried out by bats with their bodies covered with pollen from previous visits to different flowers, which would favor the effectiveness of cross-pollination. This hypothesis could be tested by analysis of capture times and by excluding bats at different times of night. Although bats were observed

TABLE 4. Dynamics of blooming among populations studied. Percentages of individuals flowering at different times of flowering season are shown. Numbers in parentheses correspond to the week number within each month. Asterisks denote significantly different values among populations compared within each region ( $P < 0.001$ ).

| Population   | % of individual plants flowering in populations | Beginning flowering | Flowering individuals with open flowers (%) |         |         |         |         | Flowering duration (days) | Mean ( $\pm$ 1 SE) no. of days of flowering per individual | Mean ( $\pm$ 1 SE) no. of flowers per individual at flowering peak |                   |                  |
|--------------|-------------------------------------------------|---------------------|---------------------------------------------|---------|---------|---------|---------|---------------------------|------------------------------------------------------------|--------------------------------------------------------------------|-------------------|------------------|
|              |                                                 |                     | up 25                                       | 25–50   | 50      | 25–50   | <25     |                           |                                                            |                                                                    | <10               | 0                |
|              |                                                 |                     |                                             |         |         |         |         |                           |                                                            |                                                                    |                   |                  |
| Tehuacán     |                                                 |                     |                                             |         |         |         |         |                           |                                                            |                                                                    |                   |                  |
| Zapotitlán-W | 59                                              | June (1)            | Jun (2)                                     | Jun (3) | Jul (3) | Jul (4) | Aug (3) | Aug (4)                   | Sep (1)                                                    | 91                                                                 | 36.75 $\pm$ 3.64  | 1.8 $\pm$ 0.28   |
| S. J. Raya-W | 20                                              | Apr (4)             | May (4)                                     | Jun (3) | Jun (4) | Jul (3) | Jul (4) | Aug (2)                   | Sep (2)                                                    | 119                                                                | 37.57 $\pm$ 6.40  | 4.1 $\pm$ 1.58   |
| Coxcatlán-W  | 13                                              | Jun (1)             | Jun (2)                                     | Jun (3) | Jun (3) | Sep (1) | Sep (2) | Sep (3)                   | Sep (3)                                                    | 105                                                                | 42.36 $\pm$ 7.33  | 4.2 $\pm$ 0.79   |
| Metzontla-M  | 100                                             | May (4)             | Jun (1)                                     | Jun (2) | Jun (3) | Aug (1) | Aug (2) | Aug (3)                   | Aug (4)                                                    | 97                                                                 | 41.58 $\pm$ 4.41  | 7.9 $\pm$ 1.55   |
| S. Lorenzo-M | 53                                              | May (2)             | May (4)                                     | Jun (3) | Jul (2) | Jul (3) | Jul (4) | Aug (4)                   | Sep (2)                                                    | 93                                                                 | 39.30 $\pm$ 6.06  | 5.7 $\pm$ 1.57   |
| Metzontla-C  | 100                                             | May (3)             | May (4)                                     | Jun (2) | Jun (3) | Aug (4) | Sep (1) | Sep (2)                   | Sep (4)                                                    | 137                                                                | 66.00 $\pm$ 4.35* | 13.7 $\pm$ 1.73* |
| Mixteca      |                                                 |                     |                                             |         |         |         |         |                           |                                                            |                                                                    |                   |                  |
| Chinango-W   | 43                                              | May (1)             | May (2)                                     | May (3) | Jul (2) | Aug (3) | Aug (4) | Sep (1)                   | Sep (2)                                                    | 99                                                                 | 40.21 $\pm$ 7.07  | 8.9 $\pm$ 2.50   |
| Chinango-M   | 22                                              | May (3)             | Jun (3)                                     | Jul (1) | Jul (2) | Aug (2) | Aug (3) | Aug (4)                   | Sep (1)                                                    | 113                                                                | 35.72 $\pm$ 7.08  | 3.1 $\pm$ 1.03   |
| Chinango-C   | 94                                              | Apr (3)             | May (3)                                     | May (4) | Jun (1) | Aug (3) | Aug (4) | Sep (1)                   | Sep (3)                                                    | 151                                                                | 56.57 $\pm$ 2.60* | 21.5 $\pm$ 2.60* |

grooming their faces after the first period of activity, it was also observed that not all the pollen becomes removed from bat faces by this action. However, direct evaluations of the effects of grooming on amounts of pollen in bat faces are yet to be performed.

Since *Stenocereus stellatus* is pollinated by bats, much movement of pollen between populations might be expected. Most of the phyllostomid bats restrict nocturnal activity to distances within 10 km of the caves where they roost (Fleming et al., 1994). However, bats of the genus *Leptonycteris*, which are among the largest bats of the Glossophaginae, have been observed by Sahley, Horner, and Fleming (1993) commuting from 30 to 60 km from their roosts to feed at cactus flowers and fruit in the Sonoran Desert.

According to information from this study, participation of bats as pollinators makes isolation by distance unlikely between the wild, managed in situ, and cultivated populations studied. In Chinango, for instance, no more than 500 m separate wild and managed in situ populations and no more than 4 km separate wild and cultivated populations. In the Tehuacán Valley, distances separating the populations studied ranged between 1 km (between managed in situ and cultivated populations from Metzontla) to 40 km (between Coxcatlán-W and San Juan Raya-W and San Lorenzo-M populations). All these distances are within the possible range of movement of bats in one night. Spatial barriers could be more important between populations from the Tehuacán Valley and La Mixteca Baja, which are separated from each other from nearly 50 km (between Chinango and Metzontla and San Juan Raya populations) to nearly 80 km (from Chinango and San Lorenzo and Coxcatlán populations). However, in the intervening areas there are populations of *S. stellatus* that could provide bridges for gene flow.

All populations of *Stenocereus stellatus* studied showed an outbreeding system of sexual reproduction. Self-pollination failed to produce fruit in all cases of both nonmanipulated and manual self-pollination. This indicates that individuals were self incompatible and that people have not selected for a self-compatible system of this plant species under cultivation. Both the role of bats as principal pollinators, which are able to transport pollen over considerable distances, as well as the pattern of traditional cultivation usually including several variants of *S. stellatus* in a single garden, increase the chances of compatible cross pollination and make selection for self-compatibility unnecessary.

The information on phenology of *Stenocereus stellatus* indicates that individuals of cultivated populations present significantly more flowers in anthesis than individuals of wild and managed in situ populations. In another study (Casas et al., 1997), it was found that cultivated populations of *S. stellatus* produce significantly more fruits per plants and that this is in part because cultivated individuals have significantly more branches, higher proportion of branches producing fruits, and, at least in the Tehuacán Valley, more fruits per branch than wild individuals, and this argument could be used for explaining differences in number of flowers per individual. Also, individuals of cultivated populations present longer flowering duration than individuals of any other population. However, the blooming season overlaps in all populations



studied for >75 d/yr, which indicates that temporal mechanisms of reproductive isolation are not operating. Another feature of phenology of *S. stellatus* that seems to be relevant in relation to population variation is the pattern of long blooming seasons for an individual plant, with few flowers available per plant. According to Sosa and Soriano (1992), this pattern of production of flowers, also observed in other columnar cacti, favors transference of pollen between individuals because flower visitors are forced to visit flowers on different individuals.

The experiments of cross-pollination between wild and cultivated phenotypes should be considered as preliminary because they included only cultivated phenotypes with the strongest signs of domestication. However, these experiments revealed that reproductive isolation may exist between some of the variants of *S. stellatus* and that this mechanism could explain, at least in part, the morphological and genetic divergence between wild, managed, and cultivated populations found by Casas (1997). More detailed studies are required to test whether barriers by pollen incompatibility occur between wild and other cultivated variants as well as between the different cultivated variants and among the wild populations themselves. Such information might allow assessment of the importance of this mechanism in explaining the patterns of variation of this species.

Artificial selection, as discussed by Casas (1997) and Casas et al. (1997), is another important factor in accounting for patterns of variation of *S. stellatus*. The sparing and enhancing of desirable phenotypes within areas influenced by human activity explain why some of the phenotypes existing in wild populations are also present in home gardens and populations managed in situ. But apart from barriers due to pollen incompatibility, the absence of the phenotypes exclusive to home gardens in wild populations could also be explained because of failures in seed germination or in the establishment of seedlings or young plants of these variants that could not survive under wild conditions. This hypothesis has yet to be tested in further studies.

Origin of the variants that are common in home gardens but absent or rare in the wild populations is uncertain. They could have arisen either by mutation or hybridization in either a wild or a cultivated population. As discussed by Casas et al. (1997), the most common form of management of this species in home gardens is by vegetative propagation. The other form of management is tolerance of seedlings in home gardens. After production of fruits people decide whether these individuals are kept or removed (Casas et al., 1997). This process seems to be crucial to explain the origin of variants that occurs exclusively in home gardens.

The variants that are exclusive to home gardens could also have arisen in wild populations but could have been eliminated by natural selection and survived only under human protection in home gardens. The hypothesis is thus that the novel variation generated and established by sexual means in home gardens has included variants that are unsuccessful and therefore rare or nonexistent in the wild (among them, variants with desirable characters for people), but that are successful in the home gardens because of human care.

Interspecific hybridization could be a source of origin

of variants exclusive to home gardens. *Stenocereus pruinosus* (Otto) Buxbaum, which may have white or yellow pulp, larger fruits, and thinner peel than *S. stellatus*, is a species that possibly hybridizes with *S. stellatus*. Although more detailed studies have to be carried out to confirm this interspecific hybridization, preliminary observations on the phenology of *S. pruinosus* and the information from this study indicate that these species are often sympatric and between April and part of June their flowering seasons may overlap in some populations. This suggests that there are neither temporal nor geographic barriers preventing hybridization between these species in the wild. Participation of bats in pollination of both *S. pruinosus* (Rojas-Martínez, 1996) and *S. stellatus* may produce interspecific hybrids, but this has yet to be demonstrated. Similarly, interspecific hybridization between *S. stellatus* and *S. treleasei* (Vaupel) Backeb., which apparently is also possible, should be examined carefully for understanding variation in *S. stellatus*.

In conclusion, *Stenocereus stellatus* shows two general strategies for reproduction. Vegetative propagation, conservative in terms of variation, may be a part of a natural strategy that permits successful phenotypes to extend in particular environments. Sexual reproduction by outbreeding, apparently obligatory, may be a part of a natural strategy to reassort variation to permit survival in the diverse environments characteristic of the area studied. People from the Tehuacán Valley and La Mixteca Baja make use of these strategies of *S. stellatus* for artificial selection when they enhance, mainly by vegetative propagation, numbers of desirable variants that have arisen by sexual reproduction. In addition, there are apparently reproductive barriers between wild and cultivated populations by pollen incompatibility. The morphological divergence between these types of populations may be maintained in part by this mechanism as well as by artificial selection against wild phenotypes in home gardens, but probably also by natural selection operating against domesticated phenotypes in the wild.

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